STUDY ON THE EFFECT OF HEAT TREATMENT ON HUMAN BONE MINERAL MICROSTRUCTURE

A Dissertation submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

PHYSICS

By

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Date:08/05/2014 Suraj Sengel Jagannath Hembram

CONTENTS

CERTIFICATE

National Institute of Technology Rourkela

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Department of Physics and Astronomy

This is to certify that, the work in the report entitled **"STUDY ON THE EFFECT OF HEAT TREATMENT ON BONE MINERAL MICROSTRUCTURE"** by **MrSurajSengelJagannathHembram,** in partial fulfilment of Master of Science degree in **PHYSICS** at the National Institute of Technology, Rourkela(Deemed University); is an authentic work carried out by him under my supervision and guidance. The work is satisfactory to the best my knowledge.

DATE:08/05/2014 Prof. D.K. BISOYI

DEPARTMENT OF PHYSICS & ASTRONOMY

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ABSTRACT

Bones fundamentally composed of organic substances, Calcium carbonate and water , actually being considered as a composite material, each component of which contributing to remarkable mechanical properties of bones. The investigation presented in this work aims to highlight the compositional changes that occur in human bone structure by thermal treatment. In addition to describe more accurately the changes to bone crystallite size and shape during an experimental heating. This study aims to elucidate more clearly the changes to bone mineral during burning using a combination of XRD (X-Ray Diffraction) and SAXS(Small Angle X-Ray Scattering) techniques. Our aim is to test the hypothesis that changes to crystallite size and shape during early stages of burning and at lower temperatures may be more readily visible using SAXS, thus opening up a new route into the investigation of the effects of heat treatment on bone mineral in biomaterials research as well as archaeological and forensic contexts.

Our results show bone crystallites begin to alter in heating to 500^0C. While the samples heated to lower temperature produce XRD traces showing little alternation to the apatite chemicals. Corresponding information obtained from XRD and SAXS shows an early subtle changes in crystal parameters.

Keywords: Composite, Organic, Inorganic, XRD, SAXS

CHAPTER-1

INTRODUCTION

1.1 HUMAN BONE AND BONE TYPES

Bones are calcified connective tissue forming the major part of skeleton of most vertebrates. Human bones are inherently complex material comprising of minerals, collagen, water, non-collagenous protein, lipids, vascular elements and cells. The bone is physiologically active and reactive tissue.

Bones are mainly classified into two types:

- Cortical(compact) bone-80%
- Cancellous(spongy) bone-20%

• COMPOSITION

Bone itself consists primarily of collagen fibres and an inorganic bone mineral in the form of small crystals. Generally, living bone contains 10 to 20% water and 60 to 70% bone mineral.The composition of the mineral component can be approximated as Hydroxyapatite (HA) with chemical formula $Ca_{10}(PO_4)_6(OH)_2$.

Composition of Adult bone:

- Water:10-20%
- Protein:20-25%
- Fat:10%
- Ash:55-60%

Composition of Ash:

- Calcium(36%)
- Phosphorous(17%)
- Magnesium(8%)
- And small amount of Sodium, Potassium, Chlorine and Fluorine.

1.3 HYDROXYAPATITE

Hydroxyapatite(HA) is a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)_2$ which can be written as $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises of two entities (http://ethesis.nitrkl.ac.in/3515/1/Thesis_final.pdf). HA material has been clinically applied in many areas of density and orthopaedics because of its excellent osteoconductive and bio active properties (Kinoshita and Maeda, 2013) . It is the main inorganic constituent of bones in humans. Nano structured HA with different morphologies like spherical, rod and fibre are found to characterise HA in different application.
STRUCTURE:

Figure 1. Structure of hydroxyapatite

Hydroxylapatite is the hydroxyl end member of the complex apatite group. The OH ion can be replaced by fluoride, chloride or carbonate, producing flouroapatite or chloroapatite (http://en.wikipedia.org/wiki/Hydroxylapatite. It crystallises in the hexagonal crystal system.

CHAPTER-2

LITERATURE SURVEY

2.1 MOLECULAR STRUCTURE

Bone is a complex, composite material with a mineral matrix commonly thought of as carbonated calcium hydroxyapatite (CHA) which has extensive heteroionic substitutions. This mineral possesses microstructural characteristics such as crystallite size, strain and stoichiometry that are critical to bone physiology and function. Estimates of bone mineral structural characteristics from X-ray diffraction data are generally subject to high uncertainties. This is due to severely overlapped diffraction peaks resulting from broad diffraction maxima and relatively low crystal symmetry. It has been suggested that more precise models of bone mineral could be derived by extrapolation from heated samples in which some crystal growth has been stimulated (Rogers and Daniels, 2002)

2.2 REASON FOR STUDY OF HEAT TREATMENT ON HUMAN BONE

Bone mineral is an important biomaterial resource. Accurate measurement of bone crystal alteration, both in structure and composition, has been a focus of biomaterial research for several years. Heat treatment has been used to deproteinate bone mineral for use in osteoimplantation, since natural hydroxyapatite with the organic matrix removed is potentially a better basis for bone grafting than synthetic materials. The usefulness of this material, however, relies on the retention of biogenic crystallite characteristics throughout the preimplantation treatment process. Changes to the biogenic composition and structure of bone mineral following heat treatment at different temperatures could

affect its efficacy in these procedures. It would be valuable to know the temperature at which crystallites begin to change, and how rapid the alteration

can be. This would allow for an optimization of the heat treatment process to maximise the removal of the organic material in bone with the minimum of disruption to the mineral. In addition, the ability to identify burning and burned bone in the forensic and archaeological records has long been an important and contentious issue.

2.3 CHARACTERISATION OF HUMAN BONE

X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) have been used to determine changes to the mineral phase of bone during heating. Results have shown that there is a generalised trend toward a more ‘perfect’ or ‘crystalline’ phase of hydroxyapatite at high temperatures. Fine-scale changes to bone ultrastructure at temperatures below 1000 degree Celcius can be difficult to detect using XRD, however, and a complementary measure of crystal change would be useful in these contexts. In the last few years, advances in technology have contributed to a resurgence in the use of small-angle X-ray scattering (SAXS) to examine crystallite nanostructure in a range of materials. This technique allows for the accurate determination of crystal size, shape, and orientation within bone independent of crystal lattice perfection. SAXS has been shown to provide
information regarding crystallite structure that is complementary to other techniques and recently has been used to characterise diagenetic change in bone.

This study aims to elucidate more clearly the changes to bone mineral during burning using a combination of XRD (or wide angle-X-ray scattering, WAXS)and SAXS techniques. We aim to test the hypothesis that changes to crystallite size and

shape during early stages of burning and at lower temperatures may be more readily visible using SAXS, thus opening up a new route into the investigation of the effects of heat treatment on bone mineral in biomaterials research as well as archaeological and forensic contexts.

CHAPTER-3

MATERIALS AND METHODS

3.1 EXPERIMENTAL

The bones used to perform the experiments were collected from local hospital(VSSUT MEDICAL COLLEGE AND HOSPITAL,BURLA, ODISHA). As a first step, in order to remove the tissue, blood and proteins, macroscopic impurities and adhered substances (including salts, ligaments and tissues stuck to the bone) the samples were cleaned with a surgical blades and forceps, then treated with a jet hot water, steams (t =100°C and p = 1 atm) in a device called AUTO CLAVE (F.Miculescus, 2011). Cortical bone samples were dried by placing them in a dessicator. The dessicator was aerated every 24 hours to release moisture or gaseous emissions, and then, the samples were cut into pieces using a jig saw. After these preliminary operations, the samples were grounded in a mortar before heat treatment, in order to remove all the organic components. Subsequently, coarse ground samples were heated in air atmosphere, at 300°C and 500^0C, at a rate of temperature rise of 5 °C/min. The temperature was maintained for 2 hours to remove the organic matrix. Thermally treated bones were crushed again using agate mortar for 30 minutes. Samples were then rinsed in deionized water to remove any organic materials and were degassed in a vacuum furnace. Finally the dried powder sample were characterized using XRD, SAXS and FTIR.

3.2 CHARACTERISATION TECHNIQUES

In order to investigate various properties of the prepared sample, it has to goes under a number of characterisation techniques. The results of which gives the information about the different optical and structural properties of sample.

- STRUCTURAL CHARACTERISATION

In order to get exact information about the crystal structure, surface morphology, particle size etc. the following characterisation techniques are applicable.

- XRD (X-Ray Diffraction)
- SAXS (Small Angle X-Ray Scattering)

- OPTICAL CHARACTERISATION

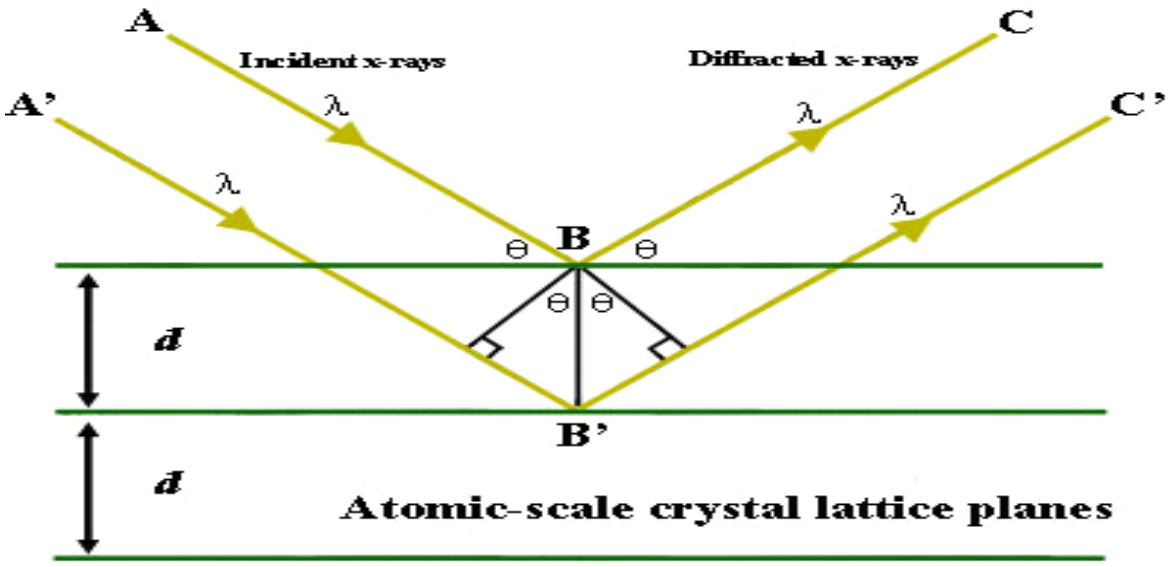
On putting the sample to following characterisation techniques gives information related to optical properties.

- FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY)

3.2.1 XRD

Upto 1895 the study of matter at the atomic level was a difficult task but the discovery of electromagnetic radiation with 1 Å wavelength, appearing at the region between gamma-rays and ultraviolet, makes it possible. As the atomic distance in matter is comparable with the wavelength of X-ray, the phenomenon of diffraction find its way through it and gives many promisable results related to the crystalline structure. The unit cell and lattices which are distributed in a regular three-dimensional way in space forms the base for diffraction pattern to occur. These lattices form a series of parallel planes with its own specific d-spacing and with different orientations exist. The reflection of incident

monochromatic X-ray from successive planes of crystal lattices when the difference between the planes is of complete number n of wavelengths leads to famous Bragg's law:



X-ray Diffraction in accordance with Bragg's Law

$$n\lambda = 2d\sin\theta$$

Where *n* is an integer 1, 2, 3….. (Usually equal 1), λ is wavelength in angstroms (1.54 Å for copper), d is interatomic spacing in angstroms, and θ is the diffraction angle in degrees. Plotting the angular positions and intensities of the resultant diffracted peaks of radiation produces a pattern, which is characteristic of the sample. The fingerprint characterization of crystalline materials and the determination of their structure are the two fields where XRD has been mostly used. Unique characteristic X-ray diffraction pattern of each crystalline solid gives the designation of "fingerprint technique" to XRD for its identification. XRD may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the interatomic distance and angle are etc. From

these points it can be concluded that X-ray diffraction has become a very important and powerful tool for the structural characterization in solid state physics and materials science.

3.2.2 SAXS

Small-angle X-ray scattering (SAXS) is a small-angle scattering (SAS) technique where the elastic scattering of X-rays (wavelength 0.1 ... 0.2 nm) by a sample which has inhomogeneities in the nm-range, is recorded at very low angles (typically 0.1 - 10°). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes, and other data (http://en.wikipedia.org/wiki/Small-angle_X-ray_scattering). SAXS is capable of delivering structural information of macromolecules between 5 and 25 nm, of repeat distances in partially ordered systems of up to 150 nm. SAXS is used for the determination of the microscale or nanoscale structure of particle systems in terms of such parameters as averaged particle sizes, shapes, distribution, and surface-to-volume ratio. The materials can be solid or liquid and they can contain solid, liquid or gaseous domains (so-called particles) of the same or another material in any combination. Not only particles, but also the structure of ordered systems (http://matxrz.net/saxs.html) like lamellae, and fractal-like materials can be studied. The method is accurate, non-destructive and usually requires only a minimum of sample preparation. Applications are very broad and include colloids of all types, metals, cement, oil, polymers, plastics, proteins, foods and pharmaceuticals and can be found in research as well as in quality control.

3.2.3 FTIR

In the region of longer wavelength or low frequency the identification of different types of chemicals is possible by this technique of infrared spectroscopy and the instrument requires for its execution is Fourier transform infrared (FTIR) spectrometer. The spectroscopy merely based on the fact that molecules absorb (S.S Pareek, 2013) specific frequencies that are characteristic of their structure termed as resonant frequencies, i.e. the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates. And the detection of energy is done on the basis of shape of the molecular potential energy surfaces, the masses of the atoms, and the associated vibronic coupling. Sometimes help of approximation techniques like Born–Oppenheimer and harmonic approximations are also taken. As each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. FTIR can be used to analyze a wide range of materials in bulk or thin films, liquids, solids, pastes, powders, fibers, and other forms. FTIR analysis can give not only qualitative (identification) analysis of materials, but with relevant standards, can be used for quantitative (amount) analysis. FTIR can be used to analyze samples up to ~11 millimeters in diameter, and either measure in bulk or the top ~1 micrometer layer. FTIR spectra of pure compounds are generally so unique that they are like a molecular "fingerprint".

CHAPTER-4

RESULTS AND DISCUSSION

4.1 X-RAY DIFFRACTION (XRD)

Figure (a) & (b) gives the X-ray diffraction pattern for samples S1 & S2 annealed at 300^{o}C and 500^{o}C respectively.

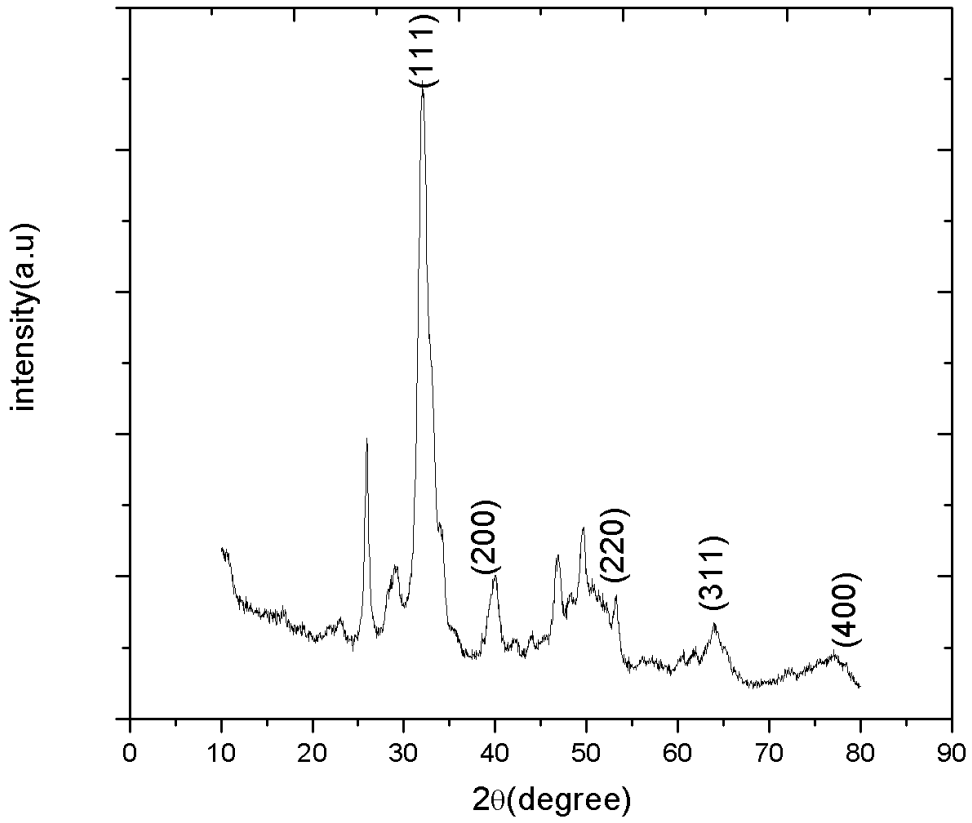


Fig: (a) XRD peak for sample annealed at 500^{0}C

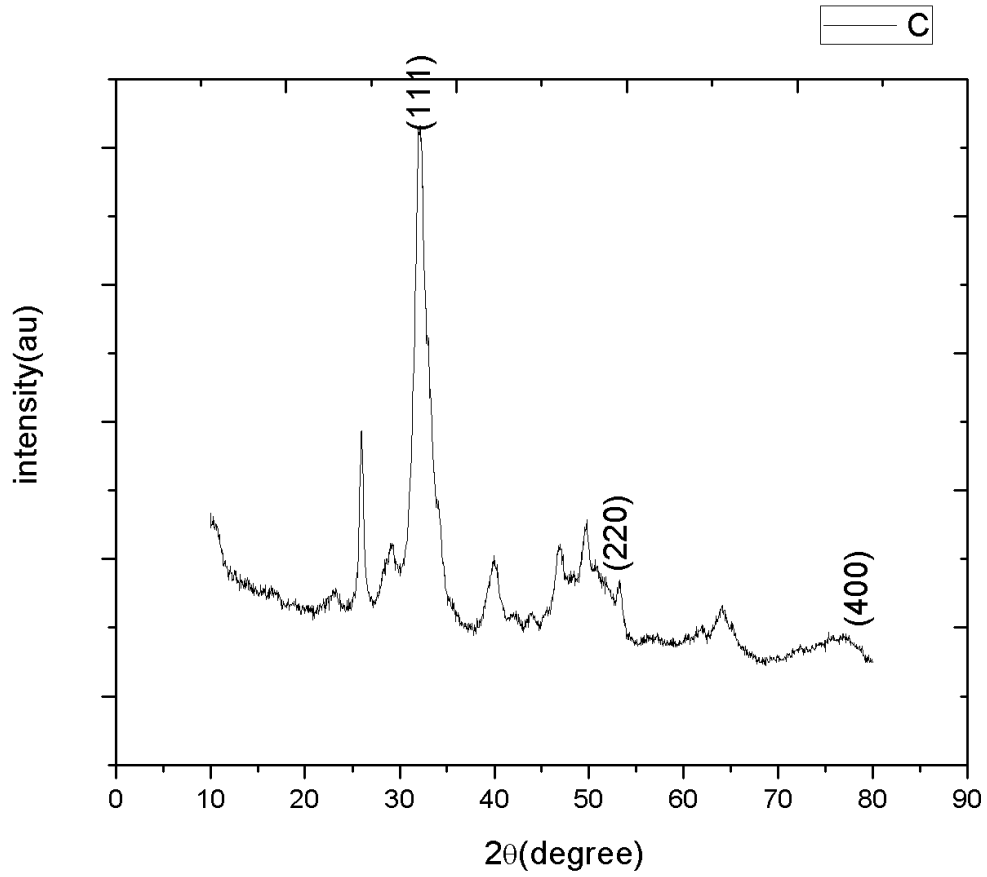


Fig: (b) XRD peak for sample annealed at 300^0C

In fig(a) as the temperature is more the CARBON content decreases and there is more formation of OXIDES generally Calcium Oxide (CaO) and due to less carbon content the powder appears to be grey in colour.

In fig(b) as the temperature is less the CARBON content is more and there is yet or less formation of OXIDES generally Calcium Oxide (CaO) and due to more carbon content the powder appears to be dark brown in colour.

And if temperature like 1000^0C were applied then there would no carbon content and the powder would be totally white which indicate the formation of oxides.

4.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

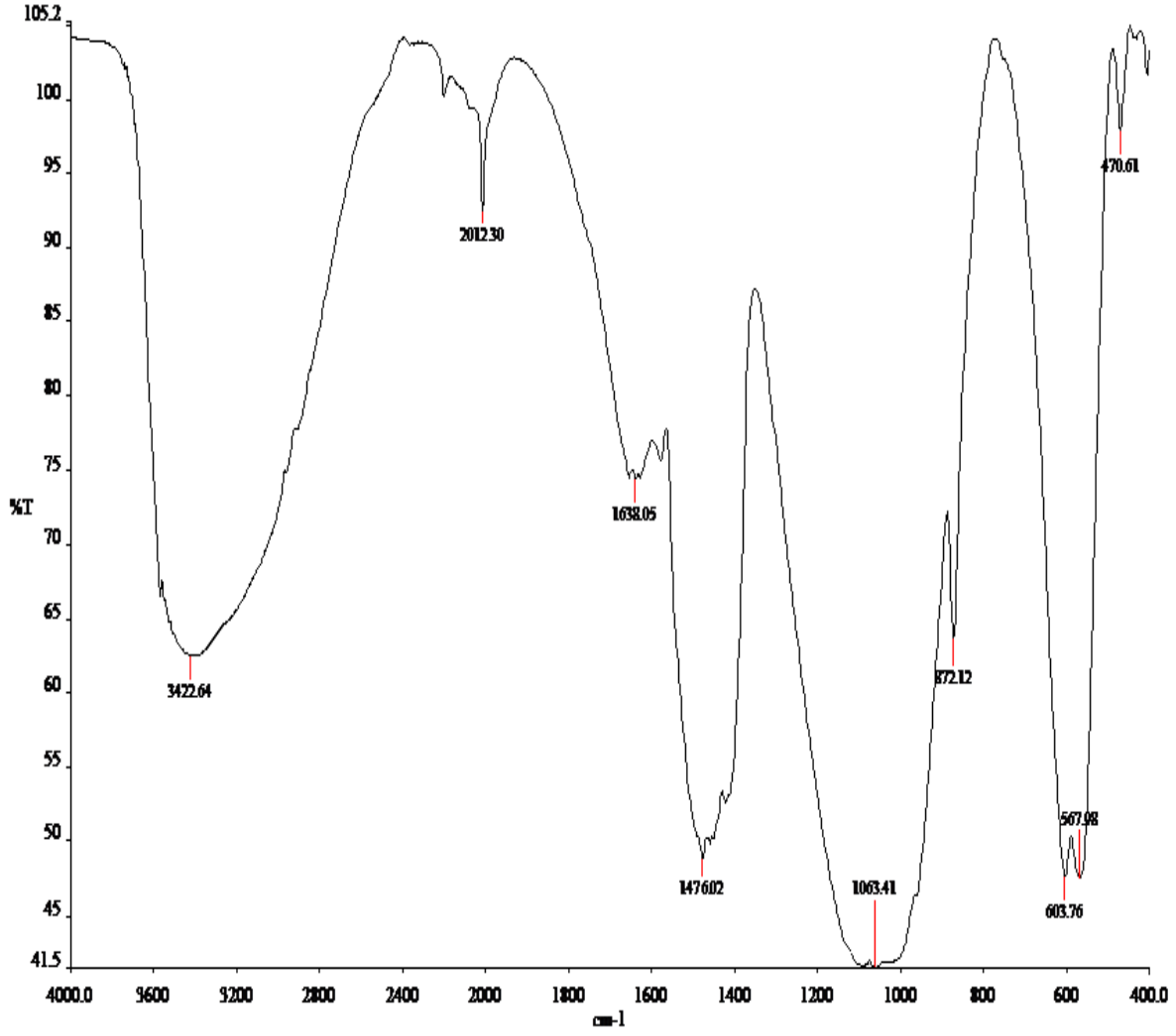


Fig: (a) FTIR peak for sample annealed at 500^0C

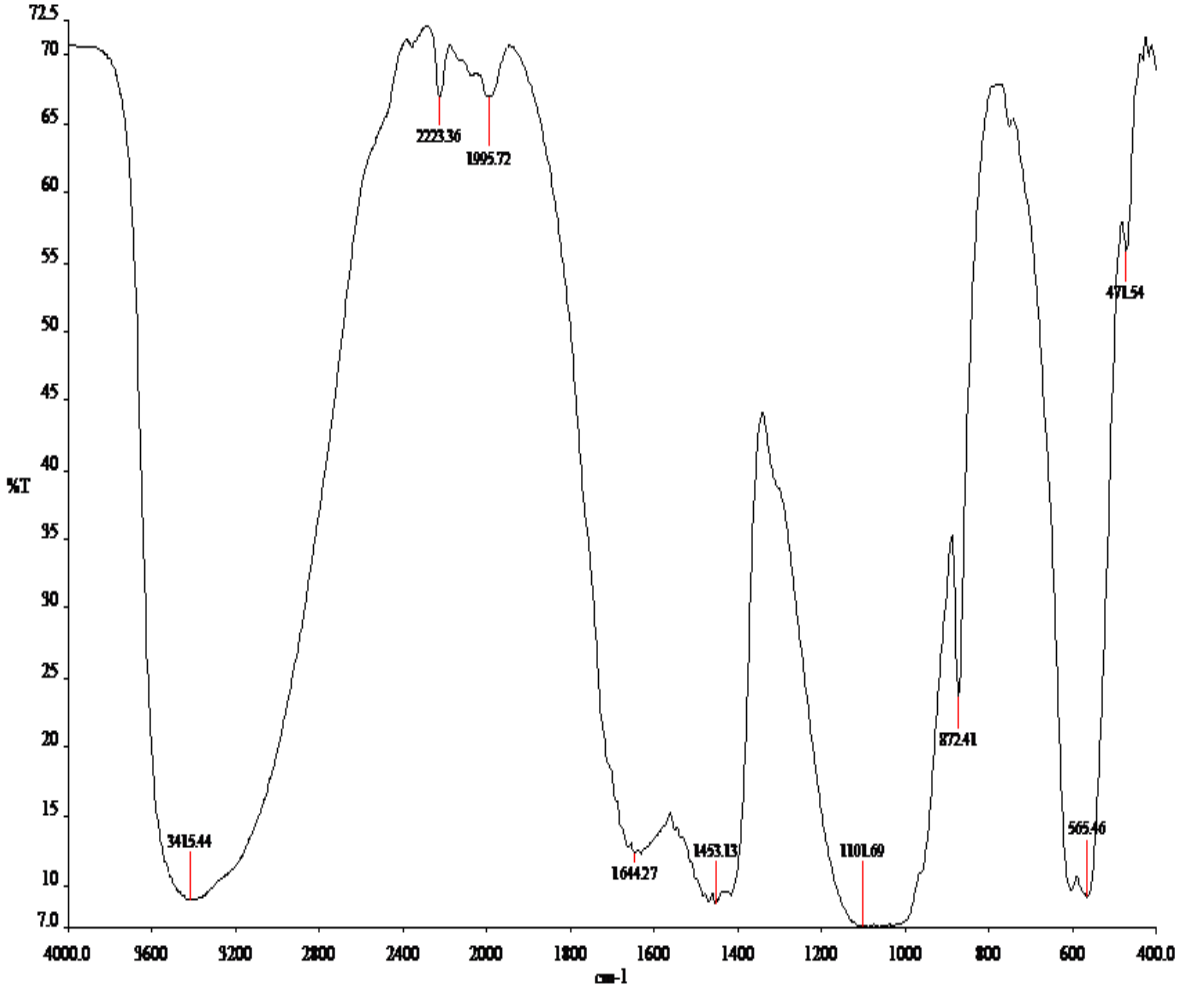


Fig: (b)) FTIR peak for sample annealed at 300^0C

Figure shows the FTIR spectrum of BONE POWDER which was aquired in the range of 400-4000 cm^{-1}.The band between the 450-700 cm^{-1} corelated to metal oxide bond. From this FTIR we can also observe that increasing the annealing temperature sharpens of the characteristic peaks for metal oxide, sugesting that, the crystalline nature increases on increasing the temperature. The peak at 800cm^{-1} -1080cm^{-1}corresponds to Si-O bond. The peaks in the range of 1400-1500cm^{-1} corresponds to the C=O bonds. The adsorbed band at 1638 cm^{-1} is assigned C-C bending vibrations. Peak in the range 3200-3500 cm^{-1} corresponds to O-H group.

4.3 SMALL ANGLE X-RAY SCATTERING (SAXS)

SAXS results show an alteration in crystal morphology with heat by recording the elastic scattering of X-Rays for very small angles.

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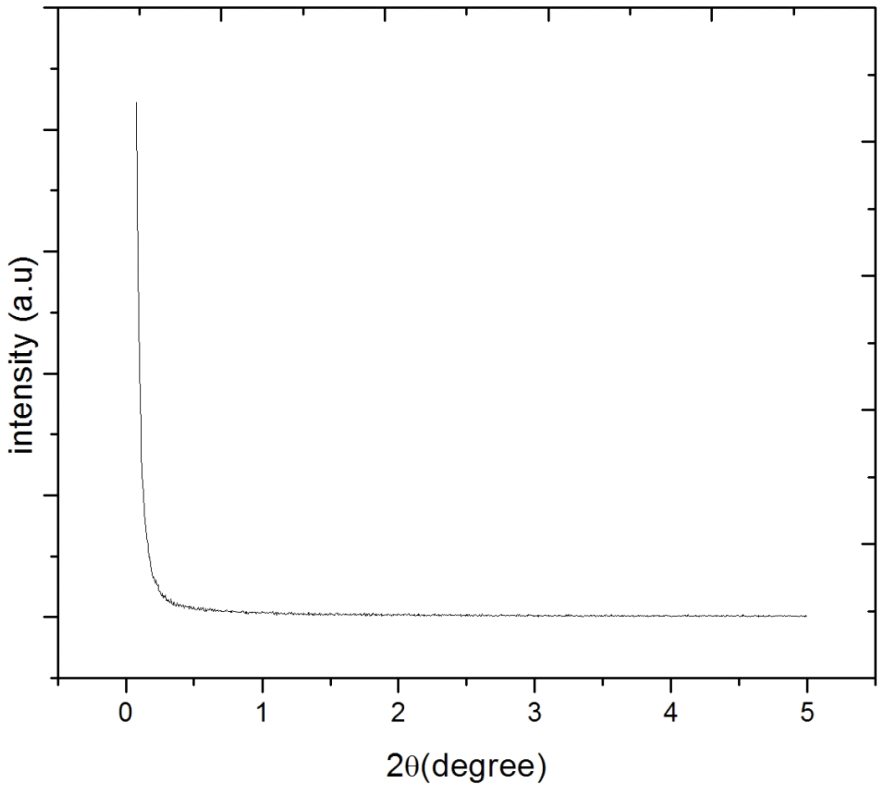
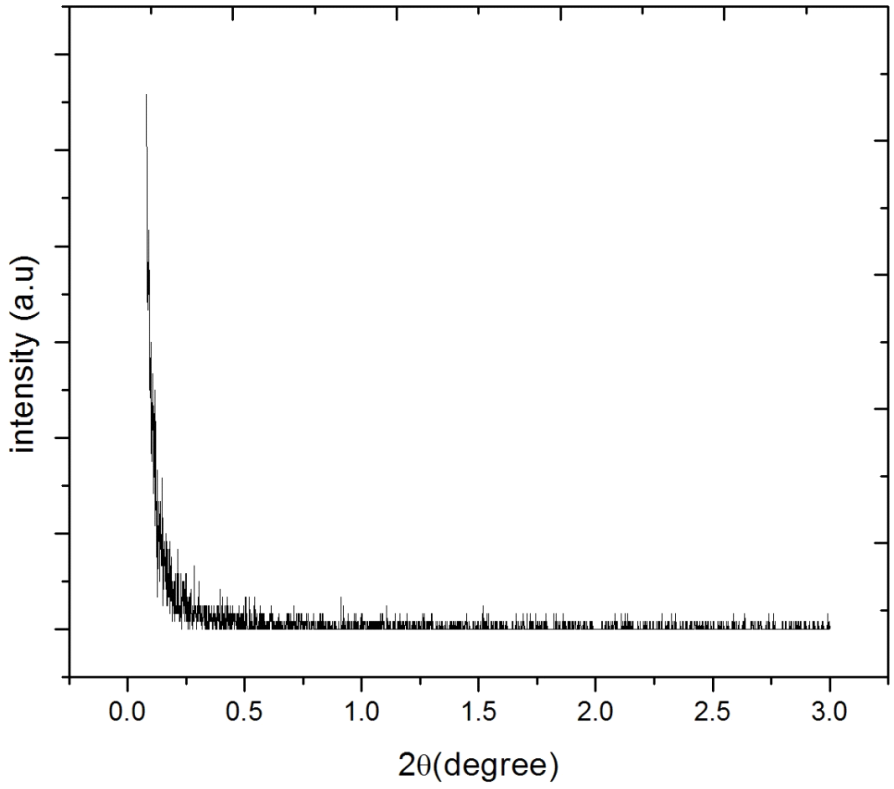
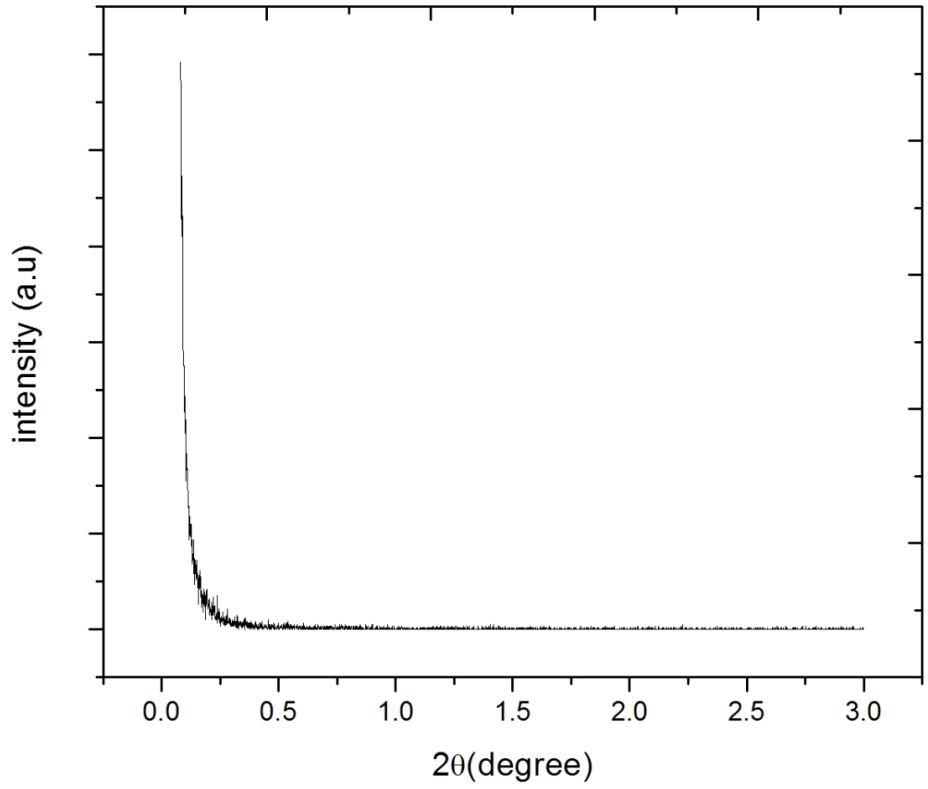



intensity (a.u)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
2θ(degree)


intensity (a.u)
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3.0
2θ(degree)

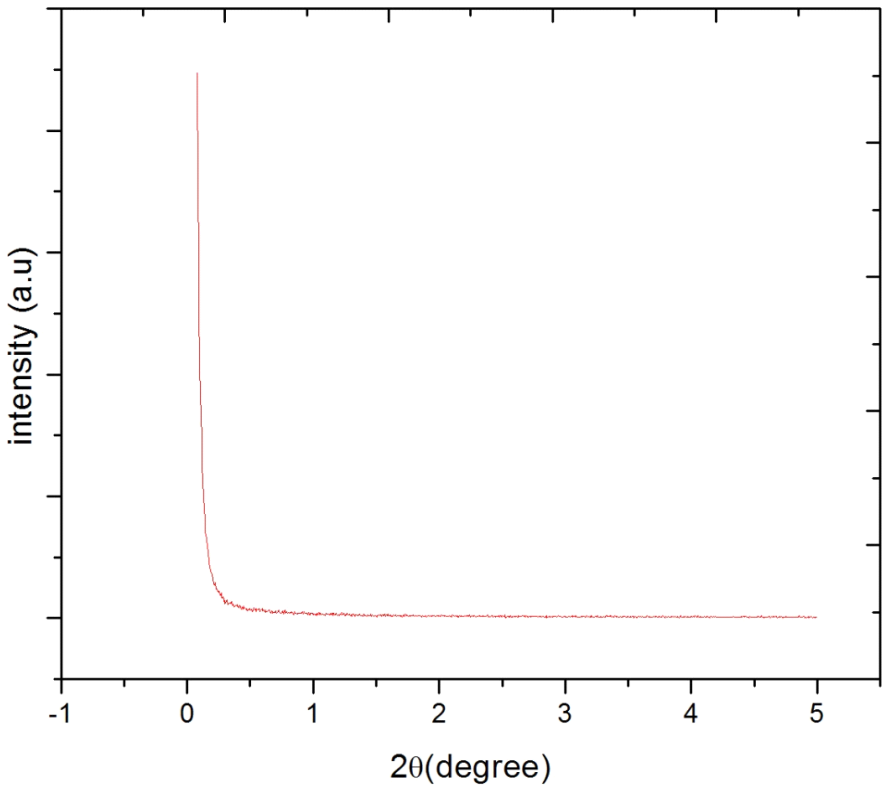

Fig: (A) shows the peak for sample annealed at 500^0C

(B) shows the peak for ethanol

(C) shows the peak for empty capillary

(D) shows the peak for sample annealed at 300^0C

CONCLUSION

XRD provides evidence in the characterisation of heated bone. Fine-scale changes in crystallite size and shape that are not measured directly using XRD. We are confident that the technique described here can be honed for use as a more accurate determinant of crystallite change during heating, thus providing an additional means of determining the effects of heat treatment on biogenic hydroxyapatite or tracing burning practices in the forensic and archaeological records.

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